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# Towards improved diagnosis of neglected zoonotic trematodes using a One Health approach<sup>☆</sup>



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## ABSTRACT

Reaching the goal of control, elimination and eradication of the Neglected Tropical Disease in a foreseeable future provides significant challenges at the ground level especially regarding helminthiasis. Helminths are still mainly diagnosed by egg identification in stool, methods with low sensitivity and for most species low specificity. Cross-sectoral collaboration with regard to zoonoses is almost non-existing and cross-validation by inter-laboratory evaluation of diagnostic tests is not a common practice. The aim of this review was to elucidate the dilemma of helminth diagnosis using zoonotic trematodes as examples. Much progress has been made improving the diagnostic sensitivity of *Opisthorchis* and *Clonorchis* using DNA-based techniques but the specificity of these tests is still a challenge due to the many most common but neglected intestinal trematodes. The burden of these diseases and ways to control them remains to be elucidated. Although efficacious drugs are available, the effectiveness of mass drug administration remains to be assessed. The importance of animal reservoirs and ways to control the diseases in animals are yet unknown. Diagnostic challenges regarding *Schistosoma japonicum* and *Schistosoma mekongi* include the many light infections and the persisting influx from the animal reservoirs. The sensitivity of the faecal based techniques suited morbidity control but will be insufficient for elimination of the helminths. More accurate diagnostic tools are required and new algorithms for detection and progression of helminth elimination will be needed. Standardized inter-laboratory test validation, inter-sectoral collaboration and establishment of an international One Health diagnostic platform, sharing best practices on diagnosis of helminth zoonoses, could all significantly contribute to control and elimination of these diseases.

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## 1. Introduction – from microscopes to molecular bands

In the second WHO report on Neglected Tropical Diseases (NTD), a detailed roadmap is outlined for control, elimination and possible eradication of the 17 identified NTD before 2020. The roadmap is a very promising effort initiating concerted action against the NTD which currently affects nearly 2 billion people Worldwide (WHO, 2013). The strategy combines five intervention strategies (i.e. preventive chemotherapy, intensive case management, vector control,

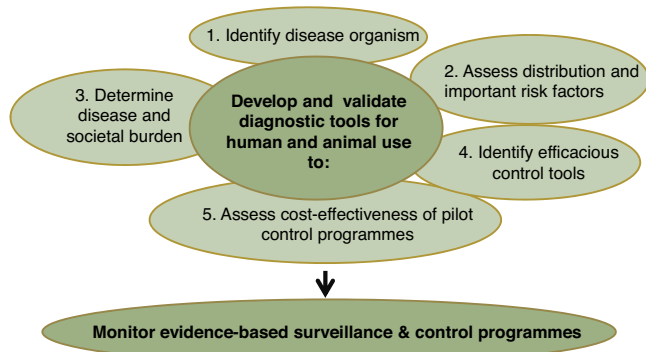
improving water and sanitation and including veterinary public health) of which preventive chemotherapy is being promoted as the most important for many of the NTD (WHO, 2013). However, the report also urges the need to refine strategies, and develop new tools and algorithms as the outlined plan causes major challenges for most existing systems. A central prerequisite for control of the NTD is accurate diagnosis and in the case of the neglected zoonoses, techniques for animal diagnosis also need to be available but as pointed out in the report by WHO (2010) major challenges exist regarding proper diagnostic and assessment tools for many of the NTD. Zoonotic trematodes like foodborne zoonotic trematodes and Asian schistosomiasis serve as good examples. Major knowledge gaps exist regarding the burden of these zoonoses and for the foodborne zoonotic trematodes even species identification is a problem. Without these, identification of the problem as well as assessment of any intervention effect will remain a challenge (Fig. 1).

Zoonotic trematodes, like most helminths, are diagnosed today in man and animals primarily by microscopy of helminth eggs in stool; a method introduced in the late 19th century.

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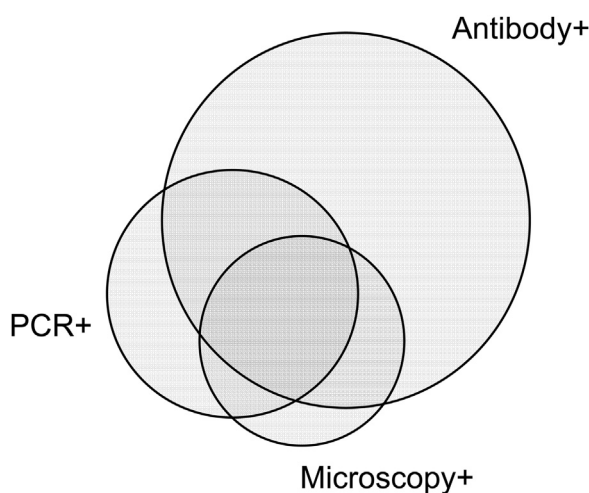


**Fig. 1.** Illustration of essential steps from parasite identification to evidence-based surveillance and control. Each step will require development and validation of appropriate diagnostic tools for both human and animal hosts.

Immunological tests, numerous and diverse, are generally less used and less validated. Only in very recent years have molecular techniques been introduced and are now being optimized and validated across country borders. As the clinical manifestations for zoonotic trematodes are non-pathognomonic, clinical diagnosis makes little sense. The lack of a reliable gold standard has been another major drawback in diagnostic advancements of these parasites. As the new tests are being evaluated against a suboptimal gold standard, the outcome of the assessment remains questionable (Fig. 2). This has been an on-going debate while trying to introduce serological tests assessing specific antibodies as well as molecular methods. While some attention has been paid to develop and improve diagnostic tools for human zoonotic trematodes, the diagnostic *ante mortem* opportunities for animal zoonotic trematodes is almost nonexistent despite a growing recognition of their importance in control and eventually eradication of these diseases. In the following an update on the diagnostic advancements and present challenges are presented for three groups of neglected zoonoses among the NTD.

### 1.1. Opisthorchiasis and clonorchiasis

The foodborne zoonotic trematodes consist of liver, lung and intestinal flukes of which the most important species parasitizing humans are the fishborne zoonotic trematodes *Opisthorchis viverrini*, *Opisthorchis felinus* and *Clonorchis sinensis* (Keiser and



**Fig. 2.** Example of combinations of test results. The accuracy of a new test (here PCR) will be wrongly perceived as low if a test with low sensitivity (here microscopy) or low specificity (here antibody detection) are used as gold standard.

Utzinger, 2009; Sithithaworn et al., 2012). Distribution of these liver flukes is focal in nature and restricted to areas where the first and second intermediate hosts are abundant. In addition, their distribution ranges are related to habit of eating raw, pickle, or undercooked fish and fishery products particularly in Southeast Asia (Grundty-Warr et al., 2012). Distribution ranges of these liver flukes do generally not overlap for example *O. viverrini* is known to occur in Thailand, Lao PDR, Cambodia and Vietnam while *C. sinensis* is endemic in China, Korea, Taiwan, Vietnam and part of Russia (Sithithaworn et al., 2012). *O. felinus* confine to Eurasia in the former Soviet Union, Kazakhstan, the Ukraine and Europe such as Italy and Germany (Pozio et al., 2013). Recent outbreak of *O. felinus* in Bolsena and Bracciano lakes in central Italy demonstrated an on-going maintenance of life cycle of this trematode in animal reservoir hosts in Europe (De Liberato et al., 2011).

The currently available diagnostic methods for liver fluke detection are still far from ideal and significant problems are seen in areas with low prevalence, light infections and co-infection with other trematodes. In addition, diagnosis problem persists due to widespread use of chemotherapy for parasite control and this may increase proportion of light infection individuals. In the following different approaches will be presented and discussed.

Conventional faecal examination as well as recovery of adult worms is still the gold standard diagnoses of *O. viverrini*, *O. felinus* and *C. sinensis*. Eggs can be detected either in faeces, bile from nasobiliary or percutaneous transhepatic biliary drainage (PTBD) during treatment of bile duct obstruction, or duodenal fluid. Adult worms may be encountered from expulsion chemotherapy (Ramsay et al., 1989; Elkins et al., 1991; Radomyos et al., 1994; Joo and Bang, 2005) or more accurately from the liver at *post mortem* examination (Sithithaworn et al., 1991). Faecal examination is routinely used for diagnosis of liver fluke infections due to non-invasiveness and ease of sample collection. Commonly used techniques include the modified formalin ether (or ethyl acetate) concentration technique (FECT) (Elkins et al., 1990), the modified Kato-Katz thick smear (Hong et al., 2003), and Stoll's dilution egg count technique (Viyanant et al., 1983). These classical coprological techniques all have major problems regarding specificity and sensitivity.

Under light microscopy, the eggs of the liver flukes are characterized by rough and thick egg shells, and are similar in shape and size to several species of food-borne trematodes belonging to the families Opisthorchiidae, Heterophyidae and Lecithodendriidae (Figs. 3 and 4). The latter two families of trematodes are collectively referred to as minute intestinal flukes (MIF) because of the small size of the adult worms compared to the liver flukes (Kaewkes, 2003; Chai et al., 2005; De and Le, 2011). Eggs of Opisthorchiidae and Heterophyidae are very similar while eggs of Lecithodendriidae do have smooth egg shell and less distinct shoulder in comparison with other two groups (Kaewkes, 2003). The marked morphological resemblance of MIF eggs with those of *O. viverrini* and *C. sinensis* increases the probability of a false positive diagnosis, with concomitant decrease in diagnostic specificity.

As the parasitological techniques rely on egg excretion, they cannot detect infection during prepatency, and they have poor sensitivity in light infections, in old chronic infections, following treatment, or when the bile duct is obstructed causing intermittent egg excretion. Repeated examinations are needed to improve diagnostic sensitivity of faecal examination. Three consecutive Kato-Katz thick smears were reported to be more sensitive than a single examination by FECT (Lovis et al., 2009). However, even with repeated stool examination using a standardized method like FECT, discrepancy remains between egg count and worm detection. For example in an autopsy study, adult *O. viverrini* were recovered from 113 of 139 (81.2%) livers but faecal examinations of the same individuals determined that only 86 cases (67%) were egg positive by FECT (Sithithaworn et al., 1991). From this study, the detection limit



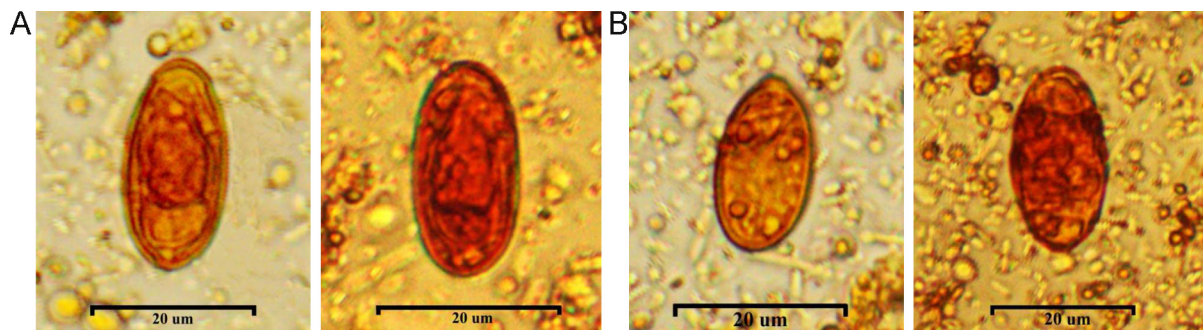


Fig. 3. Trematode eggs often encountered in endemic community of opisthorchiasis. Possibly Lecithodendriid eggs (A) and unknown trematode eggs (B).

by conventional faecal examination methods was estimated to be 20 worms or approximately 1000 eggs per gram (epg). Therefore, light infections are likely to be underdiagnosed and conventional stool examination methods may underestimate the prevalence by as much as 20% (Sithithaworn et al., 1991). Although there is evidence of density-dependent fecundity, in general there is a linear relationship between faecal egg count and worm burden. Preliminary results of a recent developed commercial stool kit (Parasep SF®) showed reduced specimen handling time, had higher sensitivity than a simple smear but had lower sensitivity than FECT (Sithithaworn, pers. commun.).

Many serological tests have been developed to overcome the sensitivity and specificity problems of the parasitological tests. These include the intradermal test, immunoelectrophoresis, indirect haemagglutination assay, indirect fluorescent antibody test and indirect enzyme-linked immunosorbent assay (indirect ELISA) (Wongratanacheewin et al., 2003; Kim et al., 2010; Hong and Fang, 2012). Indirect ELISA is often preferred for antibody detection but its sensitivity and specificity vary, mainly because of the complexity of the antigen. Crude adult somatic extracts from both *O. viverrini* and *C. sinensis* have been used for ELISA, both providing higher sensitivities than faecal examination (Poopyruchpong et al., 1990; Wongsaroj et al., 2001; Hong and Fang, 2012). Additionally, excretory/secretory (ES) antigens from adult worms have been used with superior or equivalent performance to crude antigen (Sirisinha et al., 1990; Choi et al., 2003). Recent attempts to produce recombinant antigen for serum antibody detection by ELISA, such as the recombinant egg shell protein (rOVESP) of *O. viverrini* and several antigens of *C. sinensis* such as propeptide of cathepsin L, glutathione S-transferases, lysophosphatidic acid phosphatase and cathepsin B showed increased diagnostic sensitivity and specificity over faecal conventional examination (Hong et al., 2000, 2002; Ruangsittichai et al., 2006; Hu et al., 2007; Chen et al., 2011; Li et al., 2011, 2012a,b). Although most of these recombinant proteins showed promise in terms of increased specificity and reduce cross reactivity, validation under field condition are still needed (Hong

and Fang, 2012). Additional drawback of antibody-based detection approach is the inability to differentiate past and present infections with these liver flukes, due to persistence of the antibody response which may remain elevated in the infected hosts for months or years after curative treatment (Hong, 1988; Ruangkunaporn et al., 1994; Johansen et al., 2010). A thorough assessment of the dynamics of antibody profiles following successful treatment in the absence of reinfections and cross infections is highly warranted.

As opposed to antibody detection antigen-based detection systems have also proven to be informative for current infection (Sirisinha et al., 1991, 1995; Chaicumpa et al., 1992). Monoclonal antibody (mAb)-based systems may offer an increase in diagnostic sensitivity, enough to detect secretory product from a few adult worms, even when eggs are not found in the stool (Sirisinha et al., 1995). Recent studies in animal models for *C. sinensis* (Mazidur Rahman et al., 2012) and *O. viverrini* (Duanngai, pers. commun.) suggest that coproantigen detection is a promising approach for future diagnosis of light infection as well as post chemotherapy evaluation. Field assessment of the sensitivity and specificity of these methods should be given high priority.

For copro DNA-based diagnosis, several target genes of both *O. viverrini* and *C. sinensis* such as repeated DNA elements (satellite DNA), internal transcribed spacer region of ribosomal DNA (ITS1) ITS2 and mitochondrial DNA have been used for conventional PCR and real time PCR diagnosis with variable sensitivity but high specificity (Wongratanacheewin et al., 2003; Hong and Fang, 2012; Qiao et al., 2012). For example, PCR-based detection of *O. viverrini* in human stools, based on a pair of primer of complementary to the repeat DNA element of *O. viverrini*, was used to detect egg DNA and, a specificity of 98% and a sensitivity of 100% was achieved in moderate-to heavy infections (>1000 epg) but in light infections (<200 epg), a considerably lower sensitivity was found (68%) (Wongratanacheewin et al., 2001, 2002). Another PCR-based study using the same target DNA for the detection of *O. viverrini* DNA in stool samples from Lao PDR yielded a relatively low sensitivity (50%) with high egg counts (>1000 epg) (Stensvold et al., 2006).

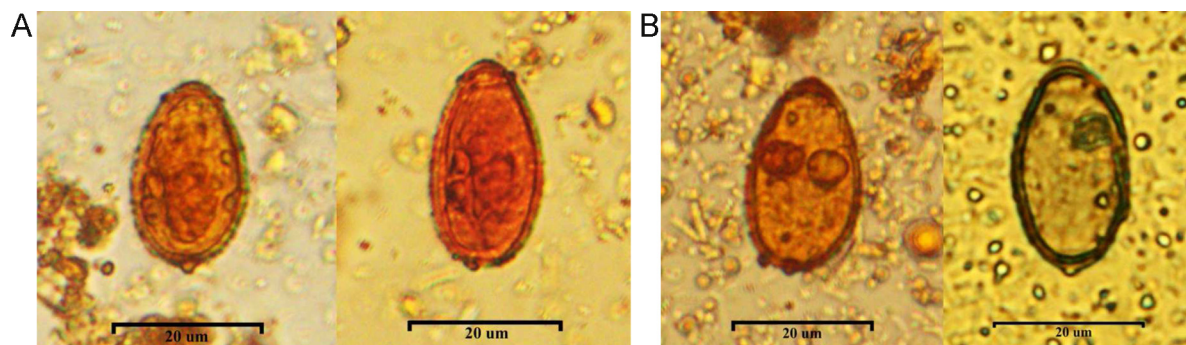


Fig. 4. *Opisthorchis viverrini* eggs (A) and degenerated *Opisthorchis* eggs (B).

Improvement of DNA preparation using cetyltrimethylammonium bromide to remove PCR inhibitors raises the sensitivity to be 79% (Duengngai et al., 2008). Interestingly, in this study, PCR-positive tests were identified in several parasite negative cases by the parasitological method (29%), indicating its advantage in diagnosis of individuals with light infection. Additional *O. viverrini*-specific primer pair was established for the PCR test and the sensitivity of the test was 10–12 ng of adult worm DNA, and three metacercariae in a fish sample (Parvathi et al., 2008). Additionally, species-specific PCR tests to distinguish between the species of liver fluke are now available for *O. viverrini* (Ando et al., 2001; Wongratanaheewin et al., 2001), *O. felinus* (Pauly et al., 2003) and *C. sinensis* (Le et al., 2006). By using real-time PCR, quantification and early detection of *C. sinensis* infection has also been demonstrated (Kim et al., 2009; Rahman et al., 2011). The loop-mediated isothermal amplification (LAMP) method was recently established for detection of *O. viverrini* and *C. sinensis* and was reported to yield higher sensitivity than conventional PCR method (Cai et al., 2010; Arimatsu et al., 2012; Le et al., 2012). These molecular diagnostic techniques are also valuable for food safety inspection as they can be used to detect other life stages of the parasites e.g. in fish products (Parvathi et al., 2007, 2008; Cai et al., 2010). A few recent studies have also been able to differentiate liver and intestinal trematodes based on copro DNA-based detection (Lovis et al., 2009; Thaenkham et al., 2011). It is anticipated that these molecular diagnostic tests, due to their high specificity, can play significant roles in anthelmintic drug efficacy evaluations, rigorous monitoring of re-infection patterns, as well as in the recognition of a potentially new endemic range of the liver flukes in Southeast Asia (Touch et al., 2009; Traub et al., 2009; Duengngai et al., 2013). However, proper validation of the copro DNA-based methods is required.

There is no doubt that molecular methods will contribute significantly towards higher sensitivity and specificity of trematode zoonoses diagnosis, but further simplification and validation of the tests as well as considerations of cost will be needed. Additionally, the importance of animal reservoirs in transmission and maintaining the infection needs urgent attendance but with the help of molecular diagnostic tools, the major problem of species identification should at least partly be solved.

### 1.2. Intestinal trematode zoonoses

Intestinal trematodes infecting humans comprise a group of 70 species belonging to 14 different families, of which more than 35 are zoonotic (Chai, 2005). Among the intestinal flukes, *Stellantchasmus falcatus*, *Haplorchis* spp. and *Procerovum* spp. have been reported to cause severe health problems in humans (WHO, 1995). These trematodes have in the past been largely overlooked, hence basic information about human and animal prevalence, transmission, their veterinary and public health burden and impact, as well as efficacy and effectiveness of control options remains to be elucidated. One of the main reasons for this lack of knowledge is the continuous use of copro-microscopy tests for human diagnosis which fail to differentiate eggs from those of the liver flukes but also because most often humans harbour multiple species infections (Chai et al., 2005; Steinmann et al., 2010).

The importance of animal reservoirs in the transmission of intestinal trematodes has received almost no attention and no standardized diagnostic tests have been established for this group of parasites. In a single study in Vietnam, Lan Anh et al. (2009) found 11 MIF species in 448 domestic animals (cats, dogs and pigs) of which 10 were reported for the first time in Vietnam. Of the 448 examined animals all harboured more than one species of MIF. From the study it was concluded that domestic animals most likely play a major role in maintaining the transmission of the intestinal trematodes.

Several copro-microscopy tests in various modifications have been used to diagnose intestinal trematode eggs. The Kato-Katz thick smear and the formalin-ether/ethyl acetate concentration techniques have been used routinely for human diagnosis (WHO, 2009). The Kato-Katz technique was also evaluated for veterinary use by Nguyen et al. (2008) but did not prove adequate. Nguyen et al. (2009) found a technique combining filtration and sedimentation useful for qualitative and quantitative detection of the small trematode eggs in domestic animals. The FLOTAC technique, a modified McMaster flotation method, has also been tested for detection of food-borne trematodes in both human and animal hosts (Cringoli et al., 2010; WHO, 2009). However, none of the methods fulfil the requirements of sensitivity and specificity (Sindberg et al., 2013). The lack of identification to species level by any copro-microscopy test is a major problem which calls for more specific methodologies.

Reliable diagnosis of the intestinal flukes requires recovery and identification of adult worms expelled from egg positive hosts. As most of the adult flukes are less than 2 mm long, recovery is tedious and identification includes special staining and morphological characterization. This does not apply to routine diagnostics but is very useful as a reference method validating new tests. Adult trematodes may easily be missed due to their size and especially if infection intensity is low. As an alternative to use worm recovery as reference test it has been suggested to use faecal samples spiked with eggs from species of interest as reference (Thaenkham et al., 2007).

Many of the characterized intestinal trematodes reside in the crypt of the villi in the small intestine, where they are attached to the mucosa and cause ulceration, inflammation and fibrosis. The main symptoms are diarrhoea, nausea, vomiting and colicky abdominal pain, all vague and non-pathognomonic symptoms, which cannot be used for proper diagnosis. However, for many of the trematodes, not even the life cycle is described and neither are the clinical manifestations (Chai et al., 2005).

Immunodiagnosis has so far been targeted towards liver flukes and intestinal trematodes have been attached as 'the unavoidable contamination'. No antibody or antigen test has been developed which can provide an accurate diagnosis of the different intestinal trematodes (WHO, 2009).

Recently, several human copro-DNA-based techniques have been developed for liver flukes and tested against intestinal flukes (Stensvold et al., 2006; Duengngai et al., 2008; Lovis et al., 2009; Sato et al., 2009; Traub et al., 2009). Sato et al. (2009) and Traub et al. (2009) concluded that their PCR method was not able to distinguish *O. viverrini* from *Haplorchis taichui* in mixed infections and generally the tests captured many more *O. viverrini* positive samples than *H. taichui*. Sato et al. (2010) hypothesized that fluctuating egg excretion from MIF could be a reason for the poor result. In a very recent experimental study, Nissen et al. (2013) showed that the egg excretion of *Haplorchis pumilio* in foxes declined to very low levels following a high excretion in the acute phase. This anti-fecundity phenomenon does most likely apply to most MIF which questions the use of tests based on egg excretion. Despite millions of people and animals being infected with MIF Worldwide the diagnosis of these helminths remains to be developed, not only for the sake of being a differential problem but also because at least some of them cause significant health problems (Chai, 2007).

### 1.3. Asian zoonotic schistosomiasis

Control strategies, mainly based on mass drug administration to humans, have been carried through for decades in most of the areas where *Schistosoma japonicum* and *Schistosoma mekongi* are still endemic. Hence the prevalence and infection intensity are often low, and parts of the human population have persisting antibodies from previous infections. This is a huge challenge

for the diagnostics. This dilemma has been discussed in a paper by Zhou et al. (2011), which also gives an overview of relevant studies.

Measuring antibodies in serum has many qualities making it suitable for use in control programmes and has been used extensively (Zhu, 2005). They are relatively cheap and easy to perform, even in large quantities. They are also often perceived as having high sensitivity even in low intensity infections. Hence, a high proportion of those who are infected will have a positive test. They also nullify the problem of egg shedding variability in stool. The main drawback is a slow rate of sero-conversion after treatment, though this rate varies between studies, even with a given assay (Alarcon de Noya et al., 1992; Rabello et al., 1997; Whitty et al., 2000; Zhu, 2005). Antibody assays may also cross react with other infections (Xu et al., 2011). Some authors argue that these shortcomings can be minimized by the use of purified antigens or detection of certain immunoglobulin isotypes (Doenhoff et al., 2004; Hamilton et al., 1998). However, none of these assays have made it into routine use.

There is an overwhelming number of different antibody assays available, differing in antigen, purity of the antigen and choice of assay system (Hamilton et al., 1998; Wu, 2002). The assays in use in large scale in recent years are indirect hemagglutination assay (IHA) and ELISA with soluble egg antigen (SEA). Variations in experimental conditions in clinical studies, and not the least a lack of a reliable diagnostic gold standard in low intensity infections results in highly variable data regarding test properties for immunodiagnostic assays (Zhou et al., 2011). Two meta analyses on clinical studies of IHA and ELISA have been published the last two years (Wang et al., 2012; Zhu et al., 2010). As expected, they showed a very wide distribution in test properties, but the analysis by Wang et al. (2012) calculated the merged sensitivity and specificity to be 76% and 73% for IHA and 85% and 50% for ELISA, respectively.

As a disease is successfully controlled, the disease prevalence in that area will decrease, but so will the positive predictive value of the diagnostic tests. Zhou et al. (2007b) and Lin et al. (2008b) used comprehensive stool examination to evaluate IHA and ELISA in field conditions. The prevalence was 7–19% in the four examined villages. Using the data from the two studies, the positive predictive value were 17–31% for the IHA and 10–25% for the ELISA, respectively. This means that 25% or less of those with a positive ELISA test and 31% or less of those with a positive IHA test were indeed infected. These figures are representative for similar studies in low prevalence settings (Zhou et al., 2011). They illustrate why some authors point out that ELISA and IHA assays currently in use are not suitable for making decisions on treatment in individuals. Instead some argue that the proportion of positives in a village can be used to determine interventions on village or area level. There is however no consensus on this matter (Doenhoff et al., 2004; Lin et al., 2008b; Wu, 2002).

In the past decade a number of rapid antibody detection assays using dipsticks or test cards have been developed. They have the advantage of easily being field-applicable and the possibility for quick decisions regarding further diagnosis and treatment. Since these assays make use of the same antigens (primarily SEA) as the conventional assays, they are prone to have the same problems with cross reactivity and persisting antibodies. Available kits include dipstick dye immunoassay (DDIA), colloidal dye immunofiltration assay (CDIFA) and dot immuno-gold filtration assay (DIGFA) (Wen et al., 2005; Xiao et al., 2005; Xu et al., 2011; Zhu et al., 2005).

Detection of antigens secreted from the parasite is in principle an interesting diagnostic alternative, as it demonstrates the presence of the parasite directly. Studied antigens include the adult worm antigens circulating anodic antigen (CAA) and circulating cathodic antigen (CCA), but also Sj31/32 and most recently soluble egg antigen (Lei et al., 2011; Van't Wout et al., 1992; Wu, 2002).

The paucity of papers on antigen detection of *S. japonicum* may in part be due to the results of a Chinese collaborative study from 1996 (Guan and Shi, 1996; Wu, 2002). Here 13 antigen detection systems were tested in parallel on a heterogeneous sera panel. The specificity was 90% or higher for nine of the assays, but only three of the assays had sensitivity above 60%, with the highest being 81%. Most antigen assays use sera instead of stool. Except for this advantage, it seems that antigen detection hitherto has failed to demonstrate a significantly superior advantage compared to even a moderate number of Kato-Katz slides. This may change as new antigens and new assays are discovered. A urine antigen test for CCA is now commercialized, but has yet to prove useful in *S. japonicum* endemic areas (Bergquist, 2013).

Microscopy of eggs in stool, using Kato-Katz thick smears have been a major method for 30 years. It is simple, cheap and provides a quantitative result as egg. However, with the Kato-Katz technique the examined amount of stool is small, and the main criticism against this method is the low sensitivity in low intensity infections. Yu et al. (1998) examined duplicate smears from seven consecutive stool samples in two villages. The proportion of individuals with at least one smear positive for *S. japonicum* increased from 42% to 68% and from 17% to 36% when one and seven stool samples were used, respectively. Lin et al. (2008a) examined a village for two consecutive years, each year using a total of six smears from two stool samples. The prevalence increased without a clear levelling-off from one to six smears. If a single smear or three smears had been used, the prevalence would have been underestimated by 55% and 25%, respectively. In both studies the underestimation was highest in those with lowest infection intensity.

Hatching test is used for diagnosis in both humans and animals, mostly in China. It utilizes a large stool sample, usually 30–50 g, and hence has potentially high sensitivity. However, the method seems difficult to standardize due to both biotic and abiotic factors. Variations in such factors may in part be the reason for the inconsistency in diagnostic performance in different studies and even within the same study (Yu et al., 2007). Hatching tests is time consuming and has not consistently shown to have higher sensitivity than Kato-Katz in humans (Hubbard et al., 2002; Zhou et al., 1998, 2011).

The search for more sensitive and specific diagnostic tests has prompted research into the use of molecular methods like PCR and the related LAMP. Several molecular targets have been evaluated in both serum and stool samples, using artificial samples and animal models. However, clinical studies are scarce and hence it is too early to tell whether the promising initial results can be conveyed to clinical use (Fung et al., 2012; Lier et al., 2009; Xu et al., 2009). The sensitivity should be substantially better than Kato-Katz to justify the increased cost.

Asian schistosomiasis is a zoonosis, but there are very few studies on diagnostic methods in animals compared to humans. Hatching test has been used to a large degree in China, but also Kato-Katz and a number of filtration/sedimentation tests (Carabin et al., 2005; Liu et al., 2012; Wang et al., 2005). Immunodiagnostic tests like ELISA have also been studied to some extent (Cheng et al., 2007; Li et al., 2012a,b; Peng et al., 2008). In general, the tests used in animals seem to be prone to the same inadequacy as their human counterparts when infection intensity is low. Two studies have shown very superior sensitivity for PCR compared to Kato-Katz and hatching test in buffalo stool (Gordon et al., 2012; Wu et al., 2010). Gordon et al. (2012) found a formalin-ethyl acetate sedimentation technique to be equally sensitive as a PCR method.

A reliable diagnostic strategy for diagnosing low intensity Asian schistosomiasis infections in humans and animals are very wished for. Effective evaluation of new strategies and tests are hindered by the lack of an accurate and precise gold standard tests.



## 2. From identification to elimination

The progress in development of sensitive and specific diagnostic tools for zoonotic trematodes has been slow, fragmented, incomplete and generally not properly validated. The need for low cost and simplicity has been the driving force rather than accuracy, which has kept these parasites neglected. Hence, routine diagnosis in most places remains with insensitive and non-specific parasitological tests for humans and no diagnosis for the animal reservoirs. From a strategic control point of view it might be relevant to group the zoonotic trematodes as they can all be targeted by the anthelmintic praziquantel but with the new WHO goal to eliminate and eventually eradicate these diseases (WHO, 2012), accuracy of test results at species level becomes crucial. There are several options which could be explored. First and foremost there is a need to establish an Internet-based platform for diagnosis of trematode zoonoses across disciplines and sectors. Stratified and specific guidelines should be agreed upon. As illustrated in Fig. 1, many different diagnostic/assessment tools will be needed depending on the assessment step. The platform should gather 'best practice' on each of the steps for both humans and animals. As the eggs of most zoonotic trematodes are indistinguishable, and clinical symptoms are non-pathognomonic, the development of tele-diagnostic might be a significant challenge. Another way, which is increasingly being used, is to collect specimens containing parasite DNA from the host of interest, either man or animal or from the environment, and send them to specialized reference laboratories for species or even strain identification. Along with the assessment of whole genome sequences of trematode zoonoses, laboratories can offer fast and accurate diagnosis within days of delivery. This would overcome the present major hindering of lack of proper golden standards and the indistinguishable co-infections. For many diagnostic purposes this would however, be unrealistically expensive, hence different algorithms are needed. For each of the steps indicated in Fig. 1, the level of accuracy and precision needed should be guiding the algorithm. Also, as a consequence of progress in control of zoonotic trematodes, fewer cases with lower infection intensities are expected, which would influence the choice of tests (Bergquist et al., 2009). Combination of tests is a well-known way of improving sensitivity and specificity despite a limited budget. For example, epidemiological surveys for schistosomiasis in China uses an initial screening with IHA or dip-stick ELISA is followed by Kato-Katz if antibody positive (Zhou et al., 2007a). In order to improve test sensitivity and specificity further, the Kato-Katz could be replaced by molecular methods or enzyme-linked immunoelectrotransfer blot assay (EITB) (Lier et al., 2009; Xu et al., 2011). Since the majority of samples will be negative, it may be possible to save cost by pooling samples and only if the mixture is positive, the individual components of the mixture would need to be tested. This strategy is widely applied in meat inspection and could easily be used in serological and molecular methods (Jia et al., 2009; Van et al., 2012).

## 3. Conclusion and the way forward

Because of the complexity of the life cycle of zoonotic trematodes which involve snail intermediate hosts, animal reservoir hosts as well as human, control and even more so elimination and eradication of these parasitic zoonoses will require a true One Health approach (Karesh et al., 2012). Although still not fully agreed on, the American Veterinary Medical Association defines One Health as: "The collaborative efforts of multiple disciplines working locally, nationally and globally to attain optimal health for people, animals and our environment" (Okello et al., 2011). This definition is in essence what is needed if zoonoses are to be controlled. Isolated sector specific control efforts against zoonoses, like

e.g. mass drug administration to human populations in endemic settings with substantial animal reservoirs, will most likely have little or no impact especially in the long term.

When moving from morbidity control to transmission control dominated by populations with low intensity and persisting antibodies from previous infections or cross-reactions from other infections, diagnostic priorities change from simplicity and low cost to high accuracy. One way to achieve higher sensitivity and specificity could be to use a combination of tests. The first test should be simple, cheap but with high sensitivity. An antibody detection test is a likely choice. Since some false positive cases may be tolerated, high specificity should be prioritized in the second test of those found positive in the first. Molecular methods or highly specific antigen or antibody detection assays should replace copro-microscopy tests.

Molecular tools for species specific diagnosis of zoonotic trematodes exist, but consensus of the algorithm to move from the option of individual diagnosis to eradication of this group of Neglected Tropical Diseases has yet to be initiated. Major challenges lies ahead as accuracy of test costs. Unlike Rapid Diagnostic Tests (RDT) for malaria which play important roles for case management and control, particularly when good quality microscopy-based diagnosis is unavailable, similar rapid tests for diagnosis of the neglected zoonotic trematodes are scarce and insufficient data are currently available to derive an evidence-based algorithm of different diagnostic methods under field condition. For disease control purpose, analysis of cost-effectiveness between mass or target-group treatment without the need for diagnosis versus selective treatment for control of parasitic disease has been raised (Evans and Guyatt, 1995; Rodgers et al., 2006). If, however, the public health sector and the veterinary and agricultural sectors could join forces and establish a One Health diagnostic platform, sharing best practices on diagnosis of trematode zoonoses or even all neglected zoonoses, much would be gained. Guidance via telecommunication, diagnosis at international central DNA-based One Health laboratories, and development of algorithms for diagnostic progression depending on the control step, will be three central elements for the success of the outlined zoonoses control.

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